

TRANSFER OF APIOSE FROM UDP-APIOSE TO 7-O-(β -D-GLUCOSYL)-APIGENIN AND 7-O-(β -D-GLUCOSYL)-CHRYSOERIOL WITH AN ENZYME PREPARATION FROM PARSLEY

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An enzyme preparation from parsley (*Petroselinum hortense Hoffm.*) catalyses the formation of apiin (7-O-[β -D-apiofuranosyl(1 \rightarrow 2) β -D-glucosyl]-5,7,4'-trihydroxyflavone) from 7-O-(β -D-glucosyl)-apigenin and UDP-apiose and of the corresponding chrysoeriol-7-apiosyl-glucoside from 7-O-(β -D-glucosyl)-chrysoeriol and UDP-apiose. Neither free apiose nor cyclic apiose-1,2-phosphate can function as a substrate for the transfer reaction.

1. Introduction

The two main flavone-glycosides of parsley are apiin (7-O-[β -D-apiofuranosyl(1 \rightarrow 2) β -D-glucosyl]-5,7,4'-tri-

hydroxyflavone) and the corresponding glycoside of chrysoeriol (5,7,4'-trihydroxy-3'-methoxyflavone) [1].

The occurrence of UDP-apiose in the UDP-sugar fraction of parsley [2] and the formation of UDP-apiose from UDP-glucuronic acid with an enzyme preparation from parsley and *Lemna minor* [3, 4] has been reported in previous work from this laboratory. We have now been able to demonstrate the transfer of apiose from UDP-apiose to 7-O-(β -D-glucosyl)-apigenin and 7-O-(β -D-glucosyl)-chrysoeriol with an enzyme preparation from parsley.

2. Methods

A solution of UDP-U- 14 C-apiose was prepared by incubation of 0.258 μ mole UDP-D- 14 C-glucuronic acid (0.05 μ Ci) [3], 0.05 μ mole NAD, 6 μ moles phosphate buffer, pH 7, and 0.37 mg *Lemna* protein [4] in a total volume of 70 μ l for 30 min at 25°. Besides unreacted UDPGA the solution also contains UDP-xylose [4]. Free apiose was prepared by addition of 20 μ l acetic acid at the end of the incubation and hydrolysis for 30 min on the boiling water bath. The solution was chromatographed on paper with solvent VII. After elution with water the apiose zone was lyophilized and apiose dissolved in 0.1 M Tris Cl, pH 7.0.

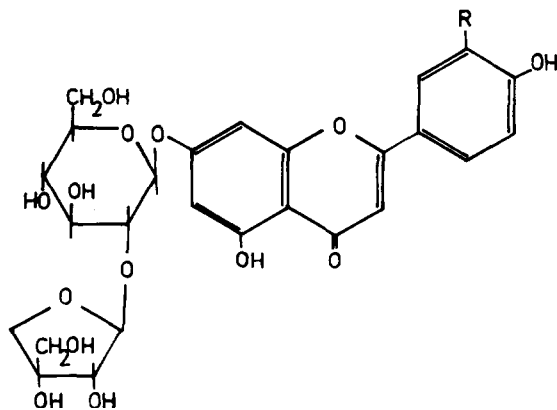


Fig. Apiin, R = H; Chrysoeriol, R = OCH₃.

Abbreviations: UDPGA, Uridinediphosphateglucuronic acid,
PVP, polyvinylpyrrolidone,
TLC, thin layer chromatography.

Cyclic apiose-1,2-phosphate [4] was obtained by adjusting the above incubation mixture to pH 8.5 at the end of the incubation and allowing it to stand at this pH for 24 hr. The solution was then chromatographed on paper with solvent IX. The radioactive zone of cyclic phosphate was detected in a scanner and after the ammonium acetate had been removed by treatment of the paper with absolute ethanol this zone was eluted with water. The eluate was lyophilized and the apiosephosphate dissolved in 0.1 M tris Cl, pH 7.0.

The enzyme from parsley was obtained by either one of two methods:

a) Five grams of leaves from 4 week old parsley plants in 10 ml 0.1 M tris Cl (pH 7.0, 0.02 M β -mercaptoethanol) were ground for 5 min with quartz sand and 1 g insoluble PVP (BASF, Ludwigshafen) in a pre-cooled mortar. The homogenate was centrifuged for 30 min at 20,000 g and the clear supernatant was used for the incubations.

b) Stems and leaves of 6 week old parsley plants were homogenized with quartz sand in a precooled mortar for 5 min in 0.025 M tris Cl, pH 7.0, and 0.01 M β -mercaptoethanol. The homogenate was squeezed through cheese cloth and centrifuged for 30 min at 20,000 g, and the clear supernatant was fractionated with solid ammoniumsulfate. The protein which precipitated between 30 to 80% saturation was dissolved in 5 ml 0.025 M tris Cl, pH 7.0, poured on a Sephadex G-25 column (18 \times 2 cm), and the protein was eluted with the same buffer. The total protein peak was used for the incubations. When the solution was stored at -20° it lost 60% of its original activity within 4 weeks.

For chromatography on paper (Schleicher Schüll 2043 b) or for TLC (silicagel G, Merck) the following solvent systems were used (in volumes):

- I. butanol/acetic acid/H₂O 4:1:5 (upper phase)
- II. butanol/H₂O 2:1 (upper phase)
- III. 15% acetic acid
- IV. ethyl acetate/methylethylketone/formic acid/H₂O 5:3:1:1
- V. chloroform/methanol/methylethylketone 9:4:1, water saturated
- VI. butanol/acetic acid/H₂O 4:1:1
- VII. ethyl acetate/pyridine/H₂O 8:2:1
- VIII. ethyl acetate/acetic acid/boric acid (saturated aqueous solution)/H₂O 20:2:1:1
- IX. ethanol/1 M NH₄-acetate, pH 7.5, 5:2

3. Results and discussion

The solution of UDP U-¹⁴C-apiose (70 μ l) (containing UDPGA-¹⁴C and UDP-xylose-¹⁴C) was incubated with 20 μ l of a solution of 0.1% 7-O-(β -D-glucosyl)-apigenin in ethyleneglycol monomethyl ether, 10 μ l 0.1 μ mole ATP in 0.1 M tris Cl, and 50 μ l of enzyme from parsley (method b, 7 mg protein/ml) at pH 7 for 30 min at 25°. After addition of 100 μ g apiin in ethanol the solution was chromatographed on paper with solvent I. One of the radioactive peaks corresponded to the R_F-value of apiin. This zone was eluted and rechromatographed on paper with solvents II and III and on silica gel with solvents IV, V, and VI. In each case the radioactivity corresponded exactly with the position of apiin. Two hundred μ g of apiin were added to each of 10 incubation mixtures (enzyme method a) at the end of incubation, and the solution was chromatographed on paper with solvent III. The radioactive zones of apiin were eluted and another 28 mg of apiin were added to the combined eluates. Apiin was then recrystallized from ethanol to constant specific activity (540 cpm/mg). The radioactivity also remained constant after apiin had been precipitated with basic lead acetate, the lead salt decomposed with H₂S and the apiin again recrystallized two times from ethanol (590 cpm/mg).

The purified radioactive apiin from several incubations was hydrolysed for 30 min on the boiling water bath with 1 M trifluoroacetic acid. The hydrolysis products were separated on paper with solvents VII and VIII. Only one radioactive product was found on the chromatograms which cochromatographed exactly with D-apiose. The radioactive apiose was further characterized by high voltage paper electrophoresis in molybdate buffer pH 5 [5].

As expected [6] the radioactive apiin was not hydrolysed by β -glucosidase (EC 3.2.1.21, Serva Entwicklungslabor, Heidelberg) whereas 7-O-(β -D-glucosyl)-apigenin was rapidly hydrolysed by this enzyme.

The highest radioactivity found in apiin under our conditions (enzyme, method b) corresponded to about 70 percent transfer of apiose to 7-O-(β -D-glucosyl)-apigenin. With heat inactivated enzyme (100° for 3 min) or with radioactive apiose or cyclic apiose-1,2-phosphate [4] instead of UDP-apiose as substrate no radioactive apiin was formed.

Incubations under the same conditions as described

above (enzyme method a) were also carried out with 7-*O*-(β -D-glucosyl)-chrysoeriol as acceptor. A radioactive zone which corresponded to chrysoeriol-7-apiosyl-glucoside [1] was detected on paper chromatograms with solvent III and rechromatographed with solvent II. The transferase activity of the enzyme preparation with 7-*O*-(β -D-glucosyl)-chrysoeriol as acceptor seems, however, to be considerably lower than with 7-*O*-(β -D-glucosyl)-apigenin, because the amounts of radioactivity in chrysoeriol-apioglucoside and apiin were 850 cpm and 10,000 cpm, respectively, under identical conditions.

Purifications of this interesting transferase for the purpose of testing its substrate specificity is in progress. Enzymatic activity for the formation of 7-*O*-(β -D-glucosyl)-apigenin from apigenin and UDP-D-glucose has also been found in extracts from parsley [7]. According to these results apiin is formed by sequential addition of glucose and apiose to apigenin.

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